

Snowpack shifts cyanobacterial community in biological soil crusts

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Abstract: Winter snowpack is an important source of moisture that influences the development of biological soil crusts (BSCs) in desert ecosystems. Cyanobacteria are important photosynthetic organisms in BSCs. However, the responses of the cyanobacterial community in BSCs to snowpack, snow depth and melting snow are still unknown. In this study, we investigated the cyanobacterial community composition and diversity in BSCs under different snow treatments (doubled snow, ambient snow and removed snow) and three snow stages (stage 1, snowpack; stage 2, melting snow; and stage 3, melted snow) in the Gurbantunggut Desert in China. In stages 1 and 2, Cyanobacteria were the dominant phylum in the bacterial community in the removed snow treatment, whereas Proteobacteria and Bacteroidetes were abundant in the bacterial communities in the ambient snow and doubled snow treatments. The relative abundances of Proteobacteria and Bacteroidetes increased with increasing snow depth. The relative abundances of Cyanobacteria and other bacterial taxa were affected mainly by soil temperature and irradiance. In stages 2 and 3, the relative abundance of Cyanobacteria increased quickly due to the suitable soil moisture and irradiance conditions. Oscillatoriales, Chroococcales, Nostocales, Synechococcales and unclassified Cyanobacteria were detected in all the snow treatments, and the most dominant taxa were Oscillatoriales and Chroococcales. Various cyanobacterial taxa showed different responses to snowpack. Soil moisture and irradiance were the two critical factors shaping the cyanobacterial community structure. The snowpack depth and duration altered the soil surface irradiance, soil moisture and other soil properties, which consequently were selected for different cyanobacterial communities. Thus, local microenvironmental filtering (niche selection) caused by snow conditions may be a dominant process driving shifts in the cyanobacterial community in BSCs.

Keywords: cyanobacterial diversity; community structure; biological soil crusts; snowpack; niche selection

1 Introduction

Winter snowfall plays a critical role in the water and energy balance in arid and semi-arid ecosystems (Zhao et al., 2016; Li et al., 2020), which are significantly affected by climate change. Small changes in temperature and precipitation may result in large changes in the amount and

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duration of snow cover (Brooks et al., 2011). It is predicted that the snowfall amount will decrease under global warming, leading to thinner snowpack in winter (IPCC, 2013; Kapnick and Delworth, 2013). However, a recent study showed that snow cover increased in winter in some regions of the Northern Hemisphere, especially after 2000 (Li et al., 2019). Biological soil crusts (BSCs) are the dominant unit of functional vegetation in arid and semi-arid regions (Rajeev et al., 2013), and they play critical roles in maintaining soil stability, improving soil structure, accumulating soil nutrients, retaining soil moisture, and reducing salt stress (Garcia-Pichel and Belnap, 1996; Hu et al., 2003a; Bowker and Belnap, 2004). However, BSCs are sensitive to changes in precipitation. Winter snowfall is an important source of moisture and influences the growth and development of BSCs in temperate desert areas, especially in Northwest China (Zhao et al., 2018).

In winter and early spring, snow cover and snow-melting processes have profound effects on microbial community composition, microbial activity, soil respiration, and carbon fixation and mineralization in BSCs in desert areas (Aanderud et al., 2013; Li et al., 2019; Ren et al., 2020). Cyanobacteria are the primary colonizers and important components of soil photosynthetic communities in BSCs (Büdel et al., 2016; Wang et al., 2020), which affect nitrogen fixation, moisture retention, soil stabilization and organic carbon accumulation in desert soils (Redfield et al., 2002; Pushkareva et al., 2018). Although many researchers have investigated the photosynthetic physiology and adaptation of BSC organisms, the responses of cyanobacterial community composition and diversity in BSCs to snowpack and snow melting are not fully understood.

Precipitation and temperature can significantly affect photoautotrophic communities in BSCs (Belnap and Lange, 2003). Generally, the response of the net photosynthesis in BSCs to the photosynthetically active photoflux density (PFD) follows a typical saturation curve. The light level required to saturate the PFD of BSCs is almost at or above 700 $\mu\text{mol photons}/(\text{m}^2\cdot\text{s})$. For Cyanobacteria in BSCs, a decrease in precipitation can lead to the functional disconnection of light-harvesting complexes from the photosystem II reaction center and can reduce cyanobacterial biomass or diversity (Lan et al., 2014; Fernandes et al., 2018). Some evidence indicates that the amount of polysaccharides in *Microcoleus vaginatus* increases with increasing light intensity (Ge et al., 2014). The net photosynthesis of BSC organisms shows a strong link to temperature. In an Antarctic dry valley, field and laboratory experiments demonstrated that the rates of net carbon (C) fixation and dark respiration in the *Nostoc commune* population depend strongly on irradiance and temperature to form *N. commune* mats with water contents higher than 30% saturation (Novis et al., 2007). Bacterial communities in undeveloped BSCs shifted significantly from their initial stages, containing few Cyanobacteria, to their late stage, dominated (27%) by *Nostoc*, *Microcoleus* and *Leptolyngbya* phylotypes during a 77-d incubation with daily freeze-thaw cycles (Schmidt and Vimercati, 2019).

In this study, we hypothesized that changes in snow conditions and stages (snowpack, melting snow and melted snow) promote soil niche differentiation (e.g., soil moisture, irradiance and soil temperature), which drives variations in cyanobacterial communities. To test this hypothesis, we investigated (1) cyanobacterial diversity in BSCs under different snow treatments and snow stages and (2) the important environmental factors driving the variation in cyanobacterial community structure in the Gurbantunggut Desert of Northwest China.

2 Materials and methods

2.1 Study area

The field experiments were conducted in the central Gurbantunggut Desert (45°03'36"N, 87°36'36"E), Xinjiang Uygur Autonomous Region, Northwest China. The Gurbantunggut Desert is the largest fixed and semi-fixed desert in China, and many areas of the desert are covered by lichen-dominated BSCs. This region has a continental, arid, temperate climate with hot and dry summers and cold winters (Su et al., 2016). The annual precipitation is 70–160 mm, falling predominantly from April to August, and the annual average potential evaporation is 2000–2800 mm. Although precipitation is low, there is a stable snow stage in winter and early spring in the

Gurbantungut Desert. Snow meltwater in early spring provides adequate moisture for BSC development in this area (Zhang et al., 2007). The annual average temperature is 5.0°C–5.7°C, and the maximum temperature is over 40.0°C. With a coverage rate of less than 30%, the natural vegetation is dominated by the small tree species *Haloxylon ammodendron* and *H. persicum*. The elevation ranges from 510 to 550 m a.s.l. (Zhang et al., 2007; Qian et al., 2008).

2.2 Experimental design

In October 2014, five fixed sample sites (10.0 m×10.0 m) were randomly established in the interdune areas among four sand dunes. Each sample site included nine plots (area of 1.5 m×1.5 m for each plot). Triplicate plots were randomly established for each of three snow treatments, including removed snow, ambient snow (natural condition) and doubled snow (Fig. 1). A 1-m buffer belt was maintained between plots to prevent the different snow treatments from influencing each other. The plots were established to avoid the inclusion of shrubs. For the plots in the removed snow treatment, transparent pieces of polycarbonate (PC) (2.0 m×2.0 m) were installed 0.6 m above the plot surface. The light transmittance of the PC was more than 95% (according to measurements of irradiance by LI-190 photoquantum sensors (Li-COR, USA)). After each snowfall, the snow on the PC board was removed and added to the plots of the doubled snow treatment. The PC boards were removed in spring, when there was no longer any snow.

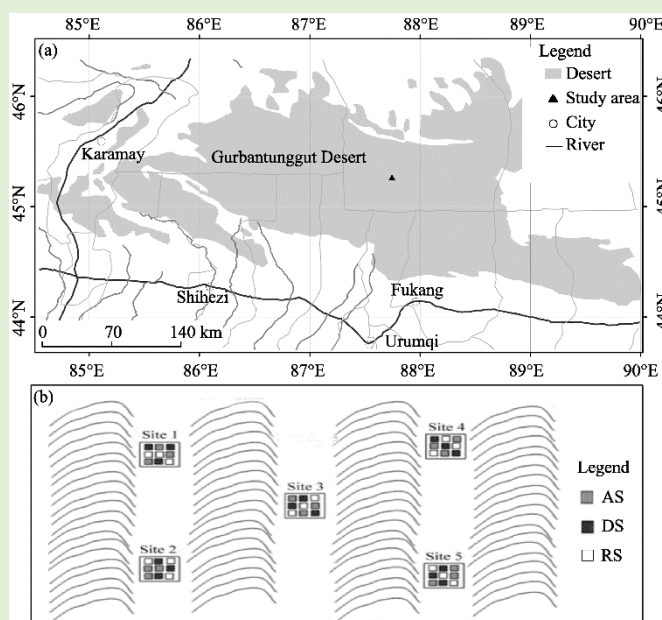


Fig. 1 Location of the study area in the Gurbantungut Desert (a) and demonstration of the experimental design (b). DS, double snow; AS, ambient snow; RS, removed snow.

2.3 Sampling methods

BSC samples were collected on 15 January (stage 1, snowpack), 1 March (stage 2, melting snow) and 15 March (stage 3, melted snow) in 2016. The snowpack stage was determined as the time at which a steady snowpack had formed. The snow melting stage began when the daytime temperature rose above 0.0°C. Stage 1 lasted from 10 December to 26 February, and stage 2 lasted from 27 February to 13 March. Stage 3 started on 13 March. To minimize spatial heterogeneity at each site, we collected triplicate BSC samples with the same snow treatment at each stage from the 0–2 cm layer and pooled to form one composite sample. Each snow treatment included five replicate samples (from five sample sites) for each stage. In total, 45 BSC samples were collected from the three snow treatments at the three snow stages. Each BSC sample was divided into two subsamples: one part of the soil sample was stored at –20°C for genomic DNA extraction, and the other part was air-dried for the measurements of soil physicochemical properties.

2.4 Soil physicochemical property analysis

Plant materials in the soil were removed by sieving with a 2-mm pore mesh. The soil organic carbon (SOC) was determined by the $K_2Cr_2O_7$ method (Walkley-Black). The total nitrogen (N) was determined by the $CuSO_4$ -Se powder diffusion method. The total phosphorus (P) was determined by the NaOH fusion-Mo Te Sc colorimetry method. The available P was determined by the 0.5 mol/L $NaHCO_3$ leaching-Mo Te Sc colorimetry method. The available N was determined by the alkali hydrolyzation-diffusion method. Soil pH and electrical conductivity were measured in a 1:5 mixture of soil and deionized water using a PHS-3C digital pH meter and a DDS-307A conductivity meter (Precision and Scientific Corp, Shanghai, China), respectively. Total salts were determined by the weight method (Chen et al., 2007; Zhang et al., 2015).

2.5 Measurements of soil moisture and photosynthetic effective radiation

Five soil moisture sensors (Decagon Devices, Pullman, WA, USA) were installed horizontally at 2 cm below the soil surface in each plot. They were connected to a Decagon EM 50 datalogger (METER, USA) that recorded the soil volumetric water content and temperature every 15 min from November 2015 to March 2016. To measure the photosynthetic effective radiation (irradiance), we installed five LI-190 photoquantum sensors (Li-COR, USA) connected to a Li-1500 datalogger (Shenzhen AKS Technology Co., China) on the surfaces of BSCs in each plot.

2.6 Soil DNA (deoxyribonucleic acid) extraction and Miseq sequencing

Soil genomic DNA was extracted from 0.5 g soil with a MOBIO Power Soil DNA Isolation Kit (MOBIO Laboratories, Carlsbad, CA, USA) using standard kit protocols. The universal forward primer 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 909R reverse primer (5'-CCCGYCAATTCMTTTRAGT-3') were used to amplify the V4 hypervariable region of the 16S rRNA gene (Xu et al., 2020). A unique 12 bp barcode was added at the 5'-end of the reverse primer, and each sample had its own unique barcode in Polymerase Chain Reaction (PCR). The PCR mixture (25 μ L) contained 1 \times PCR buffer, 1.5 mM $MgCl_2$, 0.4 mM of each deoxynucleoside triphosphate, 1.0 μ M of each primer, and 0.5 U Ex Taq (Takara Bio) and 10 ng soil genomic DNA. The PCR amplification program included initial denaturation at 94°C for 3 min, followed by 30 cycles of 94°C for 40 s, 56°C for 60 s, and 72°C for 60 s, and a final extension at 72°C for 10 min. The PCR product size was approximately 394 bp.

Two PCRs were conducted for each sample and combined after PCR amplification. PCR products were purified using a SanPrep DNA Gel Extraction Kit (Sangon Biotech, China). All the purified PCR products were mixed in equimolar amounts, used for library construction with a TruSeq DNA kit, and sequenced using an Illumina MiSeq sequencer.

2.7 Sequence data analysis

Sequence processing, clustering, taxonomic assignment and biodiversity calculations were performed with the QIIME pipeline (<http://qiime.org/>). First, sequences were demultiplexed, and primer and barcode sequences were removed. The sequences with high quality (length higher than 350 bp, without ambiguous 'N' bases, and average base quality score higher than 30) were used for downstream analyses. To compare the relative differences among samples, we selected 15,770 sequences per sample for bacteria randomly according to the rarefaction curve of the bacterial sequence data. Operational taxonomic units (OTUs) were generated by an open-reference OTU selection protocol, where sequences were clustered against the Greengenes database at a 97% similarity cutoff. Taxonomic assignments were performed using the Ribosomal Database Project (RDP) Classifier program (<http://rdp.cme.msu.edu/>) with a confidence cutoff of 80%. The original sequencing reads were deposited into the National Center for Biotechnology Information (NCBI) database (SRA accession: PRJNA490878).

Based on the taxonomic results, we resampled the cyanobacterial sequences from the bacterial sequences. To further determine the composition of cyanobacterial species, we selected representative sequences of the first 125 OTUs and blasted against the NCBI database. Then, 158 reference cyanobacterial sequences of known or higher taxonomic ranks from BSCs in deserts were

selected. The representative sequences of the OTUs in this study and the reference cyanobacterial sequences were aligned using CLUSTALX (1.83) and trimmed at each end to the same length. A neighbor-joining tree was constructed for the aligned sequences using the maximum composite likelihood model in MEGA 5.0 (Auckland, New Zealand). According to the phylogenetic tree (Fig. S1), we chose the highest Basic Local Alignment Search Tool match to the reference cyanobacterial species to represent the taxonomy of the OTU (Zhang et al., 2016).

2.8 Statistical analysis

We calculated the cyanobacterial community dissimilarity based on Bray-Curtis distance using OTU tables as the input data (Rui et al., 2015). The OTU number (based on a 97% similarity cutoff) and alpha diversity metrics (Observed-species, Chao 1) were produced for each sample in Quantitative Insights Into Microbial Ecology using the OTU table (Bokulich and Mills, 2013). The relative abundances of cyanobacteria at the phylum, order and OTU levels were determined by the percentage of the number of cyanobacterial sequences to the total bacterial sequence reads. Repeated-measures ANOVA (SPSS 13.0) was performed to test the differences in soil physicochemical properties (organic C, total N, total P, total K, available N, available P, available K, pH, electrical conductivity, total salts, soil moisture, soil temperature and irradiance), diversity indices (observed OTUs and Shannon-Wiener diversity) and relative abundances at the order and OTU levels among the different snow treatments and stages. When significant differences in different treatments and stages were observed, multiple comparisons were performed with the least significant difference (LSD) test. Pearson's correlation coefficients (two-tailed) among physicochemical properties were calculated using SPSS 13.0 prior to canonical redundancy analysis (RDA) to eliminate those variables that might distort the results due to their autocorrelations, at $P < 0.01$ level. Considering the results of the correlation analysis, organic C and total K were chosen to represent the nutrient group, while pH, total salts and electrical conductivity were selected to represent the soil salinity group. Soil moisture, soil temperature and irradiance were also included as meteorological factors.

The relative abundances of Cyanobacteria were square root-transformed prior to the analyses. PERMANOVA tests based on the Bray-Curtis distance measures were performed to test the differences in cyanobacterial community composition among the different snow treatments and stages using PAST (<http://folk.uio.no/ohammer/past/>). The correlations among cyanobacterial community dissimilarity and environmental variables were evaluated using the (partial) Mantel test with PASSaGE (<http://www.passagesoftware.net/>). To explore the relationships between the cyanobacterial community (based on OTU data) and environmental factors, we performed RDA with the rda function using the vegan package with the selected environmental variables and Hellinger-transformed data from the cyanobacterial OTU tables as the input data. The significance of environmental variables and RDA axes were determined by the envfit function using vegan in R (Borcard et al., 2011).

3 Results

3.1 Soil physicochemical properties under different snow treatments

In the Gurbantunggut Desert, the ambient snow treatment received approximately 250 (± 50) mm ($n=15$) of snowfall in the winter of 2015 and early spring of 2016. The ambient snow treatment also experienced some periods that were relatively free from snow, especially at the end of the winter season. Stage 1 was from 10 December 2015 to 26 February 2016. During this stage, the soil temperature ranged from -6.3°C to -2.9°C in the doubled snow treatment, from -7.5°C to -2.8°C in the ambient snow treatment, and from -22.7°C to 5.4°C in the removed snow treatment. No freeze-melt cycles were observed in any of the three snow treatments. In January, the snow depths in the ambient snow and doubled snow treatments were approximately 250 and 500 mm, respectively, while the removed snow treatment received little snow. Stage 2 was from 27 February 2016 to 13 March 2016; the soil temperature under the doubled snow, ambient snow and removed snow treatments varied from -5.5°C to 11.9°C , -4.9°C to 8.5°C and -8.5°C to 22.1°C , and there were

10, 6 and 15 freeze-melt cycles, respectively. Stage 3 began on 13 March 2016, and there were no occurrences of snow cover or freeze-melt cycles under the doubled snow, ambient snow and removed snow treatments.

The soil moisture ($P<0.01$), soil temperature ($P<0.01$) and irradiance ($P<0.01$) were all significantly influenced by the snow treatments, snow stages and their interactions. In stages 1 and 2, the soil moisture in the doubled snow and ambient snow treatments was significantly higher than that in the removed snow treatment ($P<0.001$). The soil moisture in the doubled snow and ambient snow treatments in stage 2 was significantly higher than that in stages 1 and 3 ($P<0.05$). The soil temperature and irradiance increased significantly from stage 1 to stages 2 and 3 ($P<0.001$). In stage 1, the soil temperature in the doubled snow and ambient snow treatments was significantly higher than that in the removed snow treatment. The irradiance in stages 1 and 2 significantly increased with decreasing snow depth ($P<0.001$). The snow treatments significantly affected SOC ($P<0.05$), total N ($P<0.05$), available K ($P<0.01$) and pH ($P<0.001$), while the snow stages significantly influenced SOC ($P<0.001$), total N ($P<0.05$) and electrical conductivity ($P<0.001$). The available P was significantly affected by the interactions of snow treatments and stages (Table 1). The SOC and total N contents in the ambient snow and removed snow treatments increased from stage 1 to stages 2 and 3. In stage 1, the available P content in the ambient snow treatment was significantly higher than that in the removed snow treatment ($P<0.05$). In stages 2 and 3, the electrical conductivity in the ambient snow treatment was higher than that in the doubled snow treatment and lower than that in the removed snow treatment (Table 2). These results indicated that both snow depth and stage influenced the soil nutrient contents (SOC, total N, available P and K), pH and electrical conductivity by altering the soil moisture and temperature; these changes may create different niches for Cyanobacteria in BSCs.

Table 1 Repeated-measures ANOVA results for the effects of snow treatments, snow stages and their interactions on the environmental properties of biological soil crusts (BSCs)

Environmental property	$F_{\text{snow treatments}} (df=2)$	$F_{\text{snow stages}} (df=2)$	$F_{\text{snow treatments} \times \text{snow stages}} (df=4)$	Environmental property	$F_{\text{snow treatments}} (df=2)$	$F_{\text{snow stage}} (df=2)$	$F_{\text{snow treatments} \times \text{snow stages}} (df=4)$
SOC	4.489*	5.995**	3.256*	pH	10.381**	2.117	0.445
Total N	5.219*	5.266*	4.849**	EC	0.947	13.675**	2.307
Total P	0.580	0.217	0.187	Total salts	3.468	0.667	1.031
Total K	1.041	2.261	0.525	Soil moisture	19.179**	129.255**	5.565**
Available N	3.290	0.580	1.979	Soil temperature	1685.724**	37.588**	409.824**
Available P	0.783	1.288	5.078**	Irradiance	3999.179**	793.259**	409.196
Available K	6.858**	0.315	0.612				

Note: SOC, soil organic carbon; EC, electrical conductivity. ** and * indicate significant correlations at the 0.01 and 0.05 levels, respectively.

Pearson's correlation analysis indicated that SOC was significantly and positively correlated with total N ($r=0.817$, $P<0.01$), total P ($r=0.357$, $P<0.05$), available P ($r=0.490$, $P<0.01$), available K ($r=0.661$, $P<0.01$) and soil temperature ($r=0.430$, $P<0.01$). In addition to SOC, the total N was positively correlated with available P ($r=0.364$, $P<0.05$) and soil temperature ($r=0.387$, $P<0.01$), and negatively correlated with available K ($r=-0.525$, $P<0.01$) (Table S1).

3.2 Cyanobacterial composition of BSCs

In BSC samples, the number of bacterial OTUs ranged from 18,619 to 18,810. The bacterial communities were composed mainly of Proteobacteria and Cyanobacteria, followed by Actinobacteria, Bacteroidetes and Acidobacteria (Fig. S2). In stages 1 and 2, Cyanobacteria were predominant in the bacterial community in the removed snow treatment and less abundant in the doubled snow and ambient snow treatments. Additionally, the relative abundance of Proteobacteria in soils was significantly higher ($P<0.05$) in the ambient snow and doubled snow treatments than in the removed snow treatment. Bacteroidetes had the highest relative abundance in the doubled snow treatment, followed by the ambient snow treatment, and it was rare in the removed snow treatment. In stage 3, Cyanobacteria dominated the bacterial community in all three snow

Table 2 Soil physicochemical properties of BSCs in different snow treatments and stages

Environmental factor	Stage 1				Stage 2				Stage 3			
	DS	AS	RS	DS	AS	RS	DS	AS	DS	AS	RS	DS
SOC (g/kg)	2.51±0.33 ^{Ba}	2.76±0.49 ^{Ba}	2.64±0.37 ^{Ba}	3.97±1.06 ^{Aa}	3.84±0.85 ^{Aa}	2.98±0.83 ^{ABb}	3.79±0.91 ^{Aa}	4.05±0.58 ^{Aa}	3.79±0.91 ^{Aa}	4.05±0.58 ^{Aa}	3.66±0.62 ^{Aa}	3.79±0.91 ^{Aa}
Total N (g/kg)	0.24±0.04 ^{Ba}	0.24±0.05 ^{Ba}	0.22±0.04 ^{Ba}	0.37±0.09 ^{Aa}	0.33±0.08 ^{Aa}	0.28±0.07 ^{Bb}	0.28±0.04 ^{Bb}	0.32±0.03 ^{Aa}	0.28±0.04 ^{Bb}	0.32±0.03 ^{Aa}	0.29±0.03 ^{Aa}	0.28±0.04 ^{Bb}
Total P (g/kg)	0.44±0.02 ^{Aa}	0.43±0.05 ^{Aa}	0.43±0.03 ^{Aa}	0.43±0.04 ^{Aa}	0.43±0.05 ^{Aa}	0.43±0.03 ^{Aa}	0.43±0.03 ^{Aa}	0.43±0.03 ^{Aa}	0.43±0.03 ^{Aa}	0.43±0.03 ^{Aa}	0.42±0.04 ^{Aa}	0.43±0.03 ^{Aa}
Total K (g/kg)	22.81±0.35 ^{Aa}	22.86±0.45 ^{Aa}	22.76±0.36 ^{Aa}	22.89±0.48 ^{Aa}	22.61±0.24 ^{Aa}	21.79±1.78 ^{Aa}	22.86±0.62 ^{Aa}	22.24±0.98 ^{Aa}	22.86±0.62 ^{Aa}	22.24±0.98 ^{Aa}	21.62±2.19 ^{Aa}	22.86±0.62 ^{Aa}
Available N (mg/kg)	31.78±7.26 ^{Aa}	36.22±6.58 ^{Aa}	28.14±10.99 ^{Aa}	22.04±8.24 ^{Ba}	22.04±4.95 ^{Ba}	24.88±7.44 ^{Aa}	26.26±4.87 ^{ABa}	27.76±4.30 ^{ABa}	26.26±4.87 ^{ABa}	27.76±4.30 ^{ABa}	33.58±10.58 ^{Aa}	26.26±4.87 ^{ABa}
Available P (mg/kg)	6.89±1.39 ^{ABb}	7.32±1.73 ^{Aa}	5.57±1.15 ^{Ab}	6.96±1.37 ^{ABb}	7.66±0.88 ^{Aa}	5.44±0.81 ^{Ab}	7.20±0.72 ^{Aa}	7.78±0.76 ^{Aa}	7.20±0.72 ^{Aa}	7.78±0.76 ^{Aa}	6.72±1.58 ^{Aa}	7.20±0.72 ^{Aa}
Available K (mg/kg)	131.90±22.30 ^{Ba}	132.10±33.30 ^{Ba}	137.10±9.50 ^{Ba}	177.00±28.30 ^{Aa}	157.70±21.40 ^{ABa}	158.70±24.30 ^{ABa}	178.40±30.90 ^{Aa}	180.70±29.30 ^{Aa}	178.40±30.90 ^{Aa}	180.70±29.30 ^{Aa}	178.10±25.50 ^{Aa}	178.40±30.90 ^{Aa}
pH (1:5)	8.62±0.11 ^{Aa}	8.55±0.13 ^{Aa}	8.57±0.07 ^{Aa}	8.51±0.12 ^{Aa}	8.48±0.13 ^{ABa}	8.44±0.16 ^{Ba}	8.36±0.09 ^{Ba}	8.36±0.10 ^{Ba}	8.36±0.09 ^{Ba}	8.36±0.10 ^{Ba}	8.28±0.06 ^{Ba}	8.36±0.09 ^{Ba}
EC (ms/cm)	75.80±11.40 ^{Aa}	81.30±14.00 ^{Aa}	83.10±15.00 ^{Ba}	65.90±10.00 ^{Ab}	70.50±2.90 ^{ABb}	84.00±14.10 ^{Ba}	67.90±5.40 ^{Ab}	68.40±4.20 ^{Ab}	67.90±5.40 ^{Ab}	68.40±4.20 ^{Ab}	95.00±13.30 ^{Aa}	67.90±5.40 ^{Ab}
Total salts (g/kg)	0.50±0.03 ^{Aa}	0.50±0.07 ^{Aa}	0.48±0.12 ^{Ba}	0.53±0.05 ^{Aa}	0.53±0.04 ^{Aa}	0.46±0.05 ^{Ba}	0.52±0.09 ^{Aa}	0.62±0.17 ^{Aa}	0.52±0.09 ^{Aa}	0.62±0.17 ^{Aa}	0.61±0.16 ^{Aa}	0.52±0.09 ^{Aa}
Soil moisture (%)	9.00±1.50 ^{Ba}	8.60±2.80 ^{Ba}	2.20±2.30 ^{Ab}	25.00±4.60 ^{Aa}	22.10±5.00 ^{Aa}	2.60±1.70 ^{Ab}	12.80±1.60 ^{Ba}	11.60±1.20 ^{Ba}	12.80±1.60 ^{Ba}	11.60±1.20 ^{Ba}	1.70±1.30 ^{Ab}	12.80±1.60 ^{Ba}
Soil temperature (°C)	-4.48±0.08 ^{Ca}	-4.76±0.05 ^{Ca}	-11.06±0.15 ^{Cb}	0.10±0.00 ^{Bb}	-2.80±0.00 ^{Bc}	1.40±0.00 ^{Ba}	3.04±0.70 ^{Ab}	2.70±1.09 ^{Ab}	3.04±0.70 ^{Ab}	2.70±1.09 ^{Ab}	7.40±0.00 ^{Aa}	3.04±0.70 ^{Ab}
Irradiance (μmol/(m²·s))	4.40±0.20 ^{Bc}	36.90±2.50 ^{Bb}	158.80±32.80 ^{Ca}	17.60±0.80 ^{Bc}	55.80±3.10 ^{Bb}	550.10±13.60 ^{Ba}	632.10±22.00 ^{Aa}	642.20±12.30 ^{Aa}	632.10±22.00 ^{Aa}	642.20±12.30 ^{Aa}	630.10±29.00 ^{Aa}	632.10±22.00 ^{Aa}

Note: Stage 1, snowpack; Stage 2, melting snow; Stage 3, melted snow; DS, double snow; AS, ambient snow; RS, removed snow. The values are means±standard deviations. Different lowercase letters in the same row indicate significant differences at the $P<0.05$ level among different snow treatments in the same stage (LSD), and different uppercase letters in the same row indicate significant differences at the $P<0.05$ level (LSD) in different stages of the same snow treatment.

treatments, and Bacteroidetes made a very small proportion of the community, suggesting the possible filtering role of irradiance within the microbial community (Fig. S2).

At the order level, the cyanobacterial communities included Oscillatoriales (212 OTUs), Chroococcales (167 OTUs), Nostocales (32 OTUs), Synechococcales (11 OTUs) and unclassified Cyanobacteria (165 OTUs) in all the treatments. Oscillatoriales and Chroococcales were the dominant taxa, accounting for 31.1%–48.7% and 22.9%–38.3% of all cyanobacterial sequences, respectively.

At the OTU level, 796 cyanobacterial OTUs were detected in BSCs. The dominant cyanobacterial OTUs in the different snow treatments were assigned to Microcoleaceae, Coleofasciculaceae, Phormidaceae, Chroococcidiopsidaceae, Chroococcaceae, Nostocaceae, Scytonemataceae, Tolypothrichaceae and Leptolynbyaceae. Sequences related to *M. vaginatus* and *Chroococcidiopsis* sp. were dominant in the BSCs, followed by *M. steenstrupii*, *Chroococcidiopsis thermalis* and *Trichodesmium contortum* (Table S2). Some OTUs related to Streptophyta were also found among the Cyanobacteria. A considerable proportion of the sequences could not be confidently classified at the genus level.

3.3 Effects of snowpack on cyanobacterial diversity and relative abundance

The Shannon-Wiener diversity of Cyanobacteria did not show significant changes among snow treatments ($P>0.05$) or stages ($P>0.05$). In stage 1, the relative abundance of Cyanobacteria was significantly higher in the removed snow treatment than in the ambient snow and doubled snow treatments ($P<0.05$). Additionally, the number of cyanobacterial OTUs was significantly lower in the doubled snow treatment than in the removed snow treatment ($P<0.05$; Fig. 2). When the snow began to melt in spring, significant increases were observed in the number of cyanobacterial OTUs in the doubled snow treatment and in the relative abundance of Cyanobacteria in the ambient snow treatment ($P<0.05$). When the snow had melted completely, the relative abundance of Cyanobacteria in the doubled snow treatment increased from stage 2 to stage 3, and no significant differences were observed among the three snow treatments in stage 3 ($P>0.05$; Fig. 2).

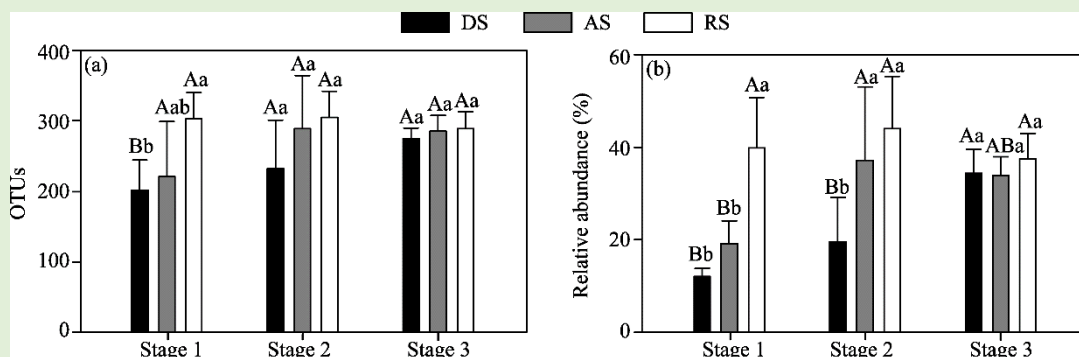


Fig. 2 Effects of snow depth and stages on (a) cyanobacterial OTUs (operational taxonomic units; richness) and (b) relative abundance in the total bacterial community. Stage 1, snowpack; Stage 2, melting snow; Stage 3, melted snow; DS, double snow; AS, ambient snow; RS, removed snow. Bars mean standard deviations. The different lowercase letters indicate significant differences at the $P<0.05$ level among different snow treatments in the same stage (LSD). The different uppercase letters indicate significant differences at the $P<0.05$ level (LSD) in different stages of the same snow treatment.

The repeated-measures ANOVA showed that the relative abundances of Oscillatoriales ($P<0.05$), Chroococcales ($P<0.01$), Synechococcales ($P<0.05$) and unclassified Cyanobacteria ($P<0.05$) changed significantly among the different snow treatments. The relative abundances of Nostocales ($P<0.05$) and Oscillatoriales ($P<0.01$) were significantly influenced by snow stage (Table 3). The interactions of snow treatments and stages significantly affected the relative abundances of Oscillatoriales ($P<0.01$) and unclassified Cyanobacteria ($P<0.01$; Table 3). In stage 1, the relative abundances of Oscillatoriales, Chroococcales, Nostocales and unclassified Cyanobacteria in the removed snow treatment significantly increased ($P<0.05$) with decreasing

snow depth. In stage 2, the relative abundances of Oscillatoriales and Chroococcales increased significantly among the three snow treatments. In stage 3, the relative abundances of most cyanobacterial orders were not significantly different among snow treatments. However, the relative abundance of Chroococcales in the removed snow treatment was significantly higher than those in the doubled snow and ambient snow treatments. The relative abundances of Oscillatoriales in the doubled snow and ambient snow treatments increased from stage 1 to stages 2 and 3, while no significant differences were observed in the relative abundances of Chroococcales, Nostocales, Synechococcales and unclassified Cyanobacteria among the different snow stages (Table 4).

Table 3 Repeated-measures ANOVA results for the effects of snow treatments, snow stages and their interactions on the relative abundances of cyanobacterial orders in BSCs

	<i>df</i>	Nostocales	Oscillatoriales	Chroococcales	Synechococcales	Unclassified Cyanobacteria
$F_{\text{snow treatments}}$	2	2.364	6.193*	6.674**	4.172*	3.223*
$F_{\text{snow stages}}$	2	4.098*	3.798*	3.395	1.231	1.646
$F_{\text{snow treatments} \times \text{snow stages}}$	4	1.630	3.798*	2.656	0.514	2.911*

Note: ** and * indicate significant correlations at the 0.01 and 0.05 levels, respectively.

In stages 1 and 2, the relative abundances of most cyanobacterial OTUs in the removed snow treatment were significantly higher than those in the doubled snow and ambient snow treatments ($P < 0.05$) and increased with decreasing snow depth, such as those of *Microcoleus vaginatus*, *M. steenstrupii*, *T. contortum*, *Symplocastrum torsivum*, *Symplocastrum californicum*, Phormidiaceae cyanobacterium, *Chroococcidiopsis* sp. CC1, *C. thermalis* and *Chroococcidiopsis* sp. CC3 (Table S2). However, in stage 3, the relative abundances of all cyanobacterial OTUs did not show significant differences among snow treatments (Table S2). The snow stages also showed significant effects on the relative abundances of some species. For example, the relative abundances of *M. vaginatus* and unknown Cyanobacteria in the doubled snow treatment showed a significant increase from stage 1 to stages 2 and 3. In the removed snow treatment, the relative abundance of *Coleofasciculus chthonoplastes* in stage 2 was significantly higher than that in stage 1 (Table S2).

3.4 Relationships between cyanobacterial community composition and environmental factors of snowpack and melting processes

According to PERMANOVA analysis, the cyanobacterial community compositions in the doubled snow and removed snow treatments were significantly different ($P = 0.025$) in stage 1. However, in stage 3, no significant differences were observed among the three treatments. The cyanobacterial community in the doubled snow treatment in stage 1 was significantly different from those in the removed snow treatment in stage 2 ($P < 0.05$) and in all three treatments in stage 3 ($P < 0.01$).

A total of 34.86% of the cumulative variation in the cyanobacterial community was explained in the RDA. The first and second axes explained 18.47% and 10.03% of the cumulative variation (Fig. 3), respectively. The correlation analysis indicated that the first axis was significantly correlated with soil moisture ($R^2 = 0.261$, $P = 0.001$) and irradiance ($R^2 = 0.184$, $P = 0.005$), while the second axis was significantly correlated with SOC ($R^2 = 0.180$, $P = 0.001$) and pH ($R^2 = 0.114$, $P = 0.016$). In stage 1, obvious snow depth effects on the cyanobacterial community compositions of BSCs in the doubled snow, ambient snow and removed snow treatments were observed, and these effects were correlated with irradiance, soil moisture and pH. In stage 2, the removed snow treatment samples were separated from the doubled snow treatment samples. The cyanobacterial community compositions were correlated with soil moisture and irradiance. Most samples in the doubled snow and ambient snow treatments in stage 2 were also positively associated with the SOC and total K contents, indicating the vital role of Cyanobacteria in C fixation and nutrient accumulation. When the snow had just melted, the cyanobacterial community compositions in the ambient snow and removed snow treatments were similar, whereas they showed some differences from that in the doubled snow treatment. A Mantel test analysis suggested that the cyanobacterial communities in different snow treatments were positively associated with soil moisture ($P = 0.00060$) and irradiance ($P = 0.00087$).

Table 4 Dominant cyanobacterial taxa and their relative abundances in the total bacterial community in different treatments and stages

Cyanobacterial taxa	Relative abundance in stage 1 (%)			Relative abundance in stage 2 (%)			Relative abundance in stage 3 (%)		
	DS	AS	RS	DS	AS	RS	DS	AS	RS
Oscillatoriales	3.78±1.94 ^{Bb}	6.78±5.44 ^{Bab}	17.59±8.21 ^{Aa}	8.78±1.81 ^{ABb}	14.52±4.32 ^{Ab}	17.68±5.69 ^{Aa}	15.88±9.54 ^{Aa}	16.52±4.53 ^{Aa}	16.16±6.17 ^{Aa}
Chroococcales	5.74±2.17 ^{Ab}	7.55±2.68 ^{Ab}	9.92±2.69 ^{Aa}	6.86±2.63 ^{Ab}	7.88±1.69 ^{Ab}	11.96±2.92 ^{Aa}	8.38±2.02 ^{Ab}	6.84±1.85 ^{Ab}	11.69±2.34 ^{Aa}
Nostocales	0.87±0.39 ^{Ab}	2.06±1.48 ^{Ab}	2.97±1.43 ^{Aa}	2.03±2.38 ^{Aa}	4.19±2.92 ^{Aa}	4.40±2.20 ^{Aa}	3.66±2.43 ^{Aa}	3.57±2.11 ^{Aa}	4.39±2.25 ^{Aa}
Synechococcales	0.22±0.22 ^{Aa}	0.30±0.27 ^{Aa}	6.28±0.27 ^{Aa}	0.37±0.30 ^{Aa}	0.43±0.23 ^{Aa}	0.68±0.16 ^{Aa}	0.41±0.29 ^{Aa}	0.45±0.28 ^{Aa}	0.65±0.22 ^{Aa}
Unclassified Cyanobacteria	1.38±1.41 ^{Ab}	2.86±2.56 ^{Ab}	8.55±4.68 ^{Aa}	3.21±3.59 ^{Aa}	6.98±4.44 ^{Aa}	10.13±7.24 ^{Aa}	6.01±4.10 ^{Aa}	5.44±2.66 ^{Aa}	4.62±3.48 ^{Aa}

Note: Stage 1, snowpack; stage 2, melting snow; stage 3, melted snow; DS, double snow; AS, ambient snow; RS, removed snow. The values are means±standard deviations. Different lowercase letters indicate significant differences at the *P*<0.05 level among different snow treatments in the same stage, and different uppercase letters indicate significant differences at the *P*<0.05 level in different stages within the same snow treatment (LSD).

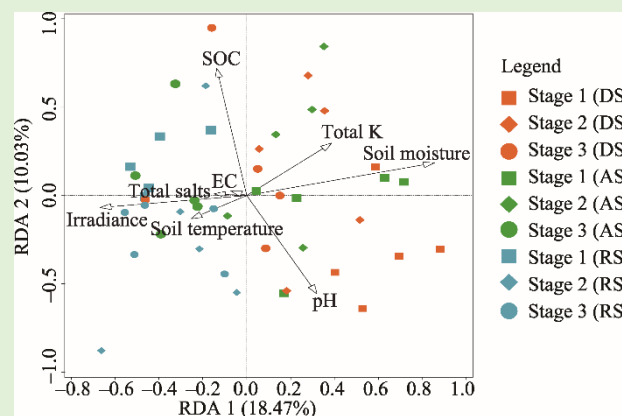


Fig. 3 RDA plots showing the relationships between cyanobacterial OTU compositions and soil physicochemical properties. RDA, canonical redundancy analysis; DS, double snow; AS, ambient snow; RS, removed snow; SOC, soil organic carbon; EC, electrical conductivity.

4 Discussion

Cyanobacteria are important autotrophic microorganisms and are primary producers of organic C and N in BSCs. Snowpack leads to changes in environmental conditions, such as soil moisture, soil temperature, irradiance and other soil properties. The present study revealed how Cyanobacteria in BSCs responded to snowpack, and soil moisture, irradiance and temperature were observed to be critical factors in shifting the structure of cyanobacterial communities.

4.1 Role of soil moisture in determining the cyanobacterial community in BSCs

As an important source of moisture in desert ecosystems, snow directly affects the soil moisture content when it melts (Zhao et al., 2018). Soil moisture showed a significant decline with reduced snow depth in stages 1 and 2. Additionally, the soil moisture levels in the doubled snow and ambient snow treatments in the different stages were significantly different (Table 2). In arid and semi-arid regions, the maximal efficiency of CO₂ fixation in BSCs remains relatively constant at optimal and greater than optimal soil water content levels but declines rapidly at lower soil water content levels (Lange, 2003). For Cyanobacteria, hydration is a critical trigger of photosynthetic activity, and hydration thresholds may determine cyanobacterial growth (Strong et al., 2013). *M. vaginatus* exhibits a form of hydrotaxis, migrating to the surface of the crust to cause visible greening during infrequent rainfall events (Rajeev et al., 2013). In stage 2, the snow began to melt, and more water became available, which promoted cyanobacterial growth. Thus, the relative abundances of dominant species (e.g., *M. vaginatus* and *Chroococcidiopsis* sp. CC1) in the doubled snow and ambient snow treatments in stage 2 were significantly higher than those in the same treatments in stage 1 (Table S2). Additionally, many other cyanobacterial OTUs showed a similar pattern to the dominant species, although those changes did not reach a significant level. As a result, the relative abundances of Oscillatoriales in the doubled snow and ambient snow treatments increased significantly from stage 1 to stage 2 (Table 4). This could be partially explained by the remarkable differences in soil moisture among the different snow treatments and stages. The differences in soil moisture among different snow treatments and stages led to variations in the relative abundances of Cyanobacteria. Although desert Cyanobacteria generally have a high capacity to withstand desiccation (Potts, 1999; Belnap and Lange, 2003), our data suggest that various cyanobacterial taxa respond differently to changes in soil moisture caused by different snow depths and stages and that these changes can result in an increase or decrease in the relative abundances of certain cyanobacterial taxa under different snow conditions.

4.2 Effects of irradiance on the cyanobacterial community in BSCs

The relative abundances of most cyanobacterial OTUs in the removed snow treatment in stage 1 were significantly higher than those in the ambient snow and doubled snow treatments ($P < 0.05$).

In stage 2, some cyanobacterial OTUs showed similar trends as in stage 1 (Table S2). Irradiance is a critical factor enhancing cyanobacterial growth under extremely dry conditions (Kiderova and Elster, 2019). As the photic zone is generally limited to a few millimeters beneath the soil surface, Cyanobacteria are significantly more abundant in the BSCs than in deeper soil layers (Garcia-Pichel et al., 2013; Steven et al., 2013). In stage 1, irradiance increased with decreasing snow depth ($P < 0.05$; Table 2). More light reached the Cyanobacteria in the removed snow treatment, which promoted cyanobacterial growth and reproduction. In contrast, in winter, the Cyanobacteria in the ambient snow and doubled snow treatments received little light ($< 55.8 \mu\text{mol}/(\text{m}^2 \cdot \text{s})$), and their growth was seriously inhibited. This resulted in lower relative abundances of Cyanobacteria in the doubled snow and ambient snow treatments than in the removed snow treatment in stages 1 and 2. In other ecosystems, such as the Tibetan Plateau and wetlands, Proteobacteria and Bacteroidetes are dominant members of the bacterial community in both the upper and deeper soil layers (Guo et al., 2015; Ade et al., 2018; An et al., 2019). The relative abundance of Proteobacteria ranges from 23% to 29% and is negatively correlated with soil depth (Wu et al., 2017; Yang et al., 2019). In alpine meadows, the relative abundance of Bacteroidetes shows a positive correlation with snow depth (Ade et al., 2018). In our results, Proteobacteria and Bacteroidetes in the doubled snow and ambient snow treatments dominated the bacterial communities in stages 1 and 2, but their abundance decreased sharply in stage 3, when the snow melted completely. These results indicated that irradiance is not necessary for the growth of Proteobacteria or Bacteroidetes.

4.3 Regulatory effect of temperature on the microbial community

Due to the thermal insulation provided by snow in winter, soil temperature regimes are always more moderate than air temperature regimes (Brooks et al., 2011). Our results indicated that snow cover increased the soil temperature in winter. When the snow melted in spring, the soil temperature in the doubled snow and ambient snow treatments was significantly lower than that in the removed snow treatment (Table 2). Soil temperature is a key factor driving microbial biomass, abundance and community structure (Yuan et al., 2012; Guo et al., 2015; Vries and Griffiths, 2018). In stages 1 and 2, the higher soil temperature and lower irradiance in the doubled snow and ambient snow treatments may have created unique niches to which Proteobacteria and Bacteroidetes may be better adapted than other taxa. Low soil temperatures and freezing conditions may lead to microbial dormancy or death (Ade et al., 2018). The soil temperature in the removed snow treatment in stage 1 was very low (-11.06°C ($\pm 0.15^\circ\text{C}$)), which may have restricted the growth of bacteria such as Proteobacteria and Bacteroidetes. However, Cyanobacteria can withstand extremely low temperatures. Viable Cyanobacteria are often detected in permafrost soils and within or on the exterior of rocks in the desert landscapes of polar regions (Vincent, 2007). Continental-scale survey of the BSC cyanobacterial communities across arid and semi-arid regions indicated that *M. vaginatus* is more thermotolerant than *M. steenstrupii* (Garcia-Pichel et al., 2013). The ability of Cyanobacteria to adapt to low temperatures partially explains why the relative abundance of Cyanobacteria in the removed snow treatment in stage 1 was higher than those in the doubled snow and ambient snow treatments (Fig. 2). In stage 3, when the snow melted completely, the soil temperature increased, and irradiance was no longer a limiting environmental factor. Under these circumstances, Cyanobacteria became dominant in bacterial communities under all snow treatments.

Climate warming leads to altered precipitation patterns, increased temperatures, and reduced snow cover (IPCC, 2013). A change in snow cover can directly or indirectly affect the soil bacterial community by, for example, promoting the dominance of heterotrophic Proteobacteria and Bacteroidetes or the dominance of autotrophic Cyanobacteria. We speculate that the long-term changes in snow cover induced by climate change may alter the dynamics of various microbial populations and their associated functions and may ultimately alter the dynamics of C and N processes by, for example, increasing heterotrophic respiration and reducing C sequestration. Further research is necessary to understand the effects of long-term changes in snow cover on the microbial community and its associated functions in BSCs.

5 Conclusions

In summary, this study revealed that changes in snowpack significantly altered bacterial community structures in BSCs of desert ecosystems. Cyanobacteria were dominant in the bacterial community of BSCs under snow removal conditions, whereas Proteobacteria and Bacteroidetes were relatively abundant under snow cover. Soil moisture, temperature and irradiance were critical factors shaping the cyanobacterial community structure in the BSCs. Cyanobacterial abundance increased quickly owing to the adequate moisture and irradiance conditions in the snow melting and melted stages. The variations in snow depth in the different snow stages promoted the formation of new niches by influencing the soil physicochemical properties (e.g., soil moisture, nutrients, temperature, pH, etc.) and irradiance, which led to changes in the cyanobacterial community of BSCs. Thus, local microenvironmental filtering (niche selection) caused by snow conditions may constitute a dominant process driving the variations in cyanobacterial communities in BSCs.

Acknowledgments

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References

- Aanderud Z T, Jones S E, Schoolmaster D R, et al. 2013. Sensitivity of soil respiration and microbial communities to altered snowfall. *Soil Biology and Biochemistry*, 57: 217–227.
- Ade L J, Hu L, Zi H B, et al. 2018. Effect of snowpack on the soil bacteria of alpine meadows in the Qinghai-Tibetan Plateau of China. *Catena*, 164: 13–22.
- An J X, Liu C, Wang Q, et al. 2019. Soil bacterial community structure in Chinese wetlands. *Geoderma*, 337: 290–299.
- Belnap J, Lange O L. 2003. *Biological Soil Crusts: Structure, Function, and Management*. Berlin: Springer, 218–260.
- Borcard D, Gillet F, Legendre P. 2011. *Numerical Ecology with R*. New York: Springer, 137–200.
- Bowker M A, Belnap J. 2004. Predictive modeling of biological soil crusts can be used as a tool for better range management. *The Bulletin of the Ecological Society of America Annual Meeting Abstracts*, 89: 58.
- Brooks P D, Grogan P, Templer P H, et al. 2011. Carbon and nitrogen cycling in snow-covered environments. *Geography Compass*, 5(9): 682–699.
- Büdel B, Dulić T, Darienko T. 2016. Cyanobacteria and algae of biological soil crusts. In: Weber B, Büdel B, Belnap J. *Biological Soil Crusts: An Organizing Principle in Drylands*. Mainz: Springer, 55–80.
- Chen Y N, Wang Q, Li W H, et al. 2007. Microbiotic crusts and their interrelations with environmental factors in the Gurbantonggut desert, western China. *Environmental Geology*, 52: 691–700.
- Fernandes V M C, Lima N M M, Roush D, et al. 2018. Exposure to predicted precipitation patterns decreases population size and alters community structure of cyanobacteria in biological soil crusts from the Chihuahuan Desert. *Environmental Microbiology*, 20(1): 259–269.
- Garcia-Pichel F, Belnap J. 1996. Microenvironments and microscale productivity of cyanobacterial desert crusts. *Journal of Phycology*, 32(5): 774–782.
- Garcia-Pichel F, Loza V, Marusenko V, et al. 2013. Temperature drives the continental-scale distribution of key microbes in topsoil communities. *Science*, 340(6140): 1574–1577.
- Ge H M, Zhang J, Zhou X P, et al. 2014. Effects of light intensity on components and topographical structures of extracellular polymeric substances from *Microcoleus vaginatus* (Cyanophyceae). *Phycologia*, 53(2): 167–173.
- Guo G X, Kong W D, Liu J B, et al. 2015. Diversity and distribution of autotrophic microbial community along environmental gradients in grassland soils on the Tibetan Plateau. *Applied Microbiology and Biotechnology*, 99(20): 8765–8776.
- Hu C X, Zhang D L, Huang Z B, et al. 2003. The vertical microdistribution of cyanobacteria and green algae within desert crusts and the development of the algal crusts. *Plant and Soil*, 257(1): 97–111.
- IPCC. 2013. *Climate Change 2013: The Physical Science Basis*. In: Stocker T F, Qin D, Plattner G K, et al. *Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge: Cambridge University Press.

- Kapnick S B, Delworth T L. 2013. Controls of global snow under a changed climate. *Journal of Climate*, 26(15): 5537–5562.
- Kiderová J, Elster J. 2019. Ecophysiology of cyanobacteria in the Polar Regions. In: Mishre A K, Tiwari D N, Rai A N. *Cyanobacteria from Basic Science to Applications*. London: Academic Press, 277–301.
- Lan S B, Wu L, Zhang D L, et al. 2014. Desiccation provides photosynthetic protection for crust cyanobacteria *Microcoleus vaginatus* from high temperature. *Physiologia Plantarum*, 152(2): 345–354.
- Lange O L. 2003. Photosynthesis of soil-crust biota as dependent on environmental factors. In: Belnap J, Lange O L. *Biological Soil Crusts: Structure, Function, and Management*. Berlin: Springer, 217–240.
- Li P, Sayer E J, Jia Z, et al. 2020. Deepened winter snow cover enhances net ecosystem exchange and stabilizes plant community composition and productivity in a temperate grassland. *Global Change Biology*, 26(5): 3015–3027.
- Li Y, Tao H, Su B D, et al. 2019. Impacts of 1.5°C and 2°C global warming on winter snow depth in Central Asia. *Science of The Total Environment*, 651: 2866–2873.
- Novis P M, Whitehead D, Gregorich E D G, et al. 2007. Annual carbon fixation in terrestrial populations of *Nostoc commune* (Cyanobacteria) from an Antarctic dry valley is driven by temperature regime. *Global Change Biology*, 13(6): 1224–1237.
- Potts M. 1999. Mechanisms of desiccation tolerance in cyanobacteria. *Europe Journal of Phycology*, 34(4): 319–328.
- Pushkareva E, Pessi I S, Namsaraev Z, et al. 2018. Cyanobacteria inhabiting biological soil crusts of a polar desert: Sør Rondane Mountains, Antarctica. *Systematic and Applied Microbiology*, 41(1): 363–373.
- Qian Y B, Wu Z N, Zhao R F, et al. 2008. Vegetation patterns and species-environment relationships in the Gurbantunggut Desert of China. *Journal of Geographical Sciences*, 18(4): 400–414.
- Rajeev L, da Rocha U N, Klitgord N, et al. 2013. Dynamic cyanobacterial response to hydration and dehydration in a desert biological soil crust. *ISMI Journal*, 7(11): 2178–2191.
- Redfield E, Barns S M, Belnap J, et al. 2002. Comparative diversity and composition of cyanobacteria in three predominant soil crusts of the Colorado Plateau. *FEMS Microbiology Ecology*, 40(1): 55–63.
- Ren Y, Zhang L, Yang K, et al. 2020. Short-term effects of snow cover manipulation on soil bacterial diversity and community composition. *Science of The Total Environment*, 741. doi: 10.1016/j.scitotenv.2020.140454.
- Schmidt S K, Vimercati L. 2019. Growth of cyanobacterial soil crusts during diurnal freeze-thaw cycles. *Journal of Microbiology*, 57(4): 243–251.
- Steven B, Gallegos-Graves L V, Belnap J, et al. 2013. Dryland soil microbial communities display spatial biogeographic patterns associated with soil depth and soil parent material. *FEMS Microbiology Ecology*, 86(1): 101–113.
- Strong C L, Bullard J E, Burford M A, et al. 2013. Response of cyanobacterial soil crusts to moisture and nutrient availability. *Catena*, 109: 195–202.
- Su Y G, Huang G, Lin Y J, et al. 2016. No synergistic effects of water and nitrogen addition on soil microbial communities and soil respiration in a temperate desert. *Catena*, 142: 126–133.
- Vincent W F. 2007. Cold tolerance in cyanobacteria and life in the cryosphere. In: Seckbach J. *Algae and Cyanobacteria in Extreme Environments* (e-book). Netherlands: Springer, 287–301.
- Vries F T D, Griffiths R L. 2018. Impacts of climate change on soil microbial communities and their functioning. *Developments in Soil Science*, 35: 111–129.
- Wang J, Zhang P, Bao J, et al. 2020. Comparison of cyanobacterial communities in temperate deserts: A cue for artificial inoculation of biological soil crusts. *Science of The Total Environment*, 745, doi: 10.1016/j.scitotenv.2020.140970.
- Wu X D, Xu H Y, Liu G M, et al. 2017. Bacterial communities in the upper soil layers in the permafrost regions on the Qinghai-Tibetan plateau. *Applied Soil Ecology*, 120: 81–88.
- Xu L, Zhu B J, Li C, et al. 2020. Development of biological soil crust prompts convergent succession of prokaryotic communities. *Catena*, 187: 104360.
- Yang L, Jiang M, Zhu W H, et al. 2019. Soil bacterial communities with an indicative function response to nutrients in wetlands of Northeastern China that have undergone natural restoration. *Ecological Indicators*, 101: 562–571.
- Yuan H Z, Ge T D, Chen C Y, et al. 2012. Significant role for microbial autotrophy in the sequestration of soil carbon. *Applied and Environmental Microbiology*, 78(7): 2328–2336.
- Zhang B C, Zhou X B, Zhang Y M. 2015. Responses of microbial activities and soil physical-chemical properties to the successional process of biological soil crusts in the Gurbantunggut Desert, Xinjiang. *Journal of Arid Land*, 7(1): 101–109.
- Zhang Y M, Chen J, Wang X Q, et al. 2007. The spatial distribution patterns of biological soil crusts in the Gurbantunggut Desert, Northern Xinjiang, China. *Journal of Arid Environments*, 68(4): 599–610.
- Zhao R M, Hui R, Wang Z L, et al. 2016. Winter snowfall can have a positive effect on photosynthetic carbon fixation and biomass accumulation of biological soil crusts from the Gurbantunggut Desert, China. *Ecological Research*, 31(2): 251–262.
- Zhao R M, Hui R, Liu L C, et al. 2018. Effects of snowfall depth on soil physical-chemical properties and soil microbial biomass in moss-dominated crusts in the Gurbantunggut Desert, Northern China. *Catena*, 169: 175–182.

Appendix

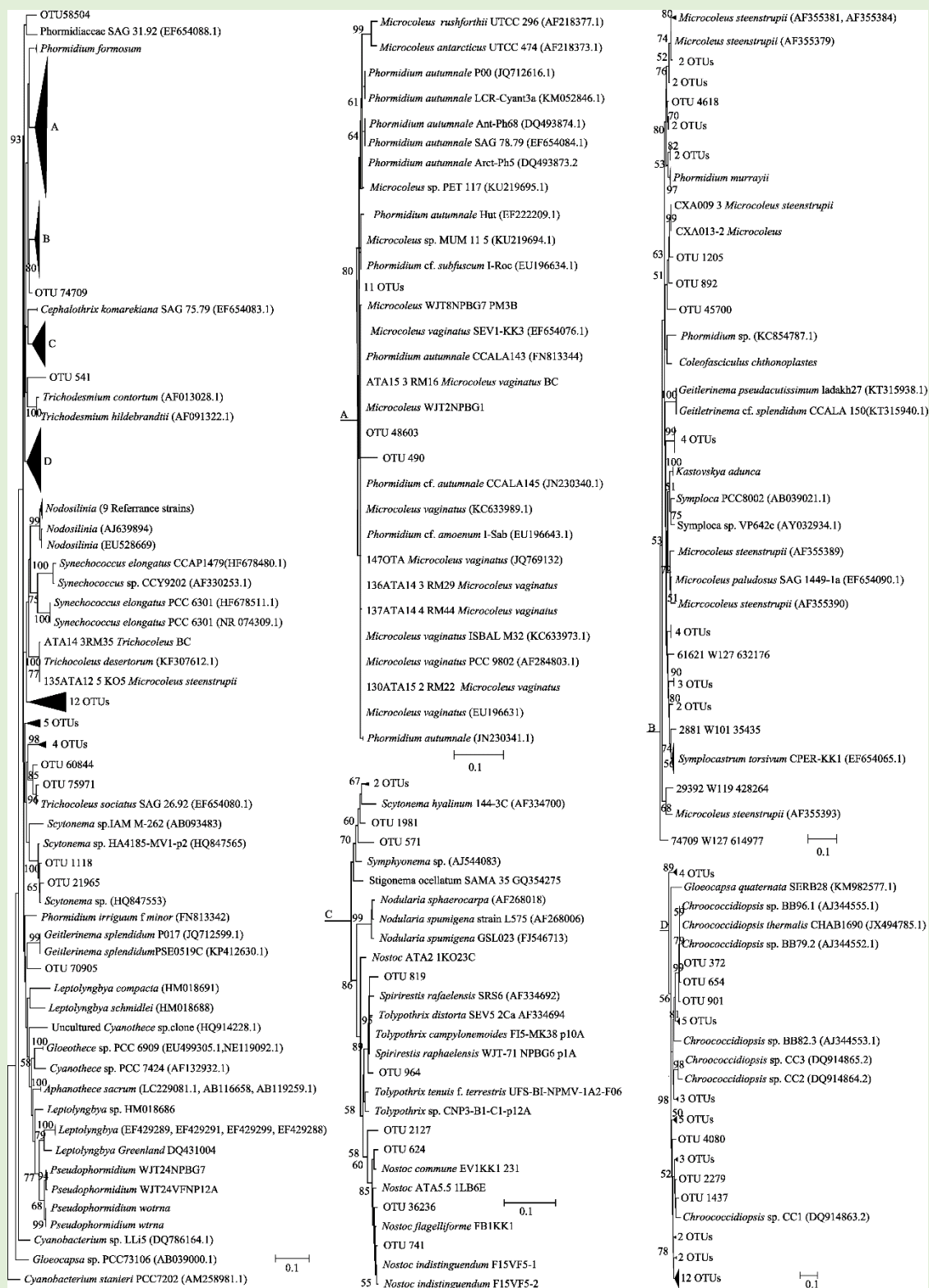


Fig. S1 Phylogenetic trees based on the 16S rRNA (ribosomal ribonucleic acid) gene representative sequences of cyanobacterial operational taxonomic units (OTUs) from biological soil crusts (BSCs) samples and reference sequences using maximum likelihood method

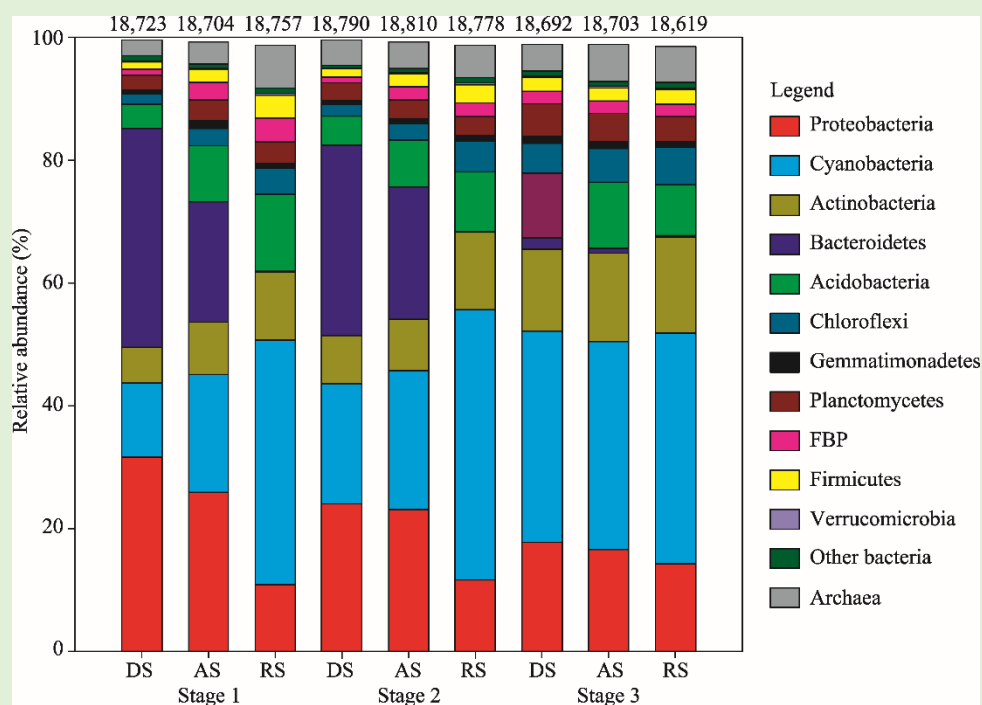


Fig. S2 Bacterial community compositions in different snow treatments and snow stages. Stage 1, snowpact; Stage 2, melting snow; Stage 3, melted snow; DS, double snow; AS, ambient snow; RS, removed snow. The data in the figure are the mean relative abundance of five replicate samples. The data at top of each bar is the total numbers of bacterial OTUs.

Table S1 Correlation coefficients among soil physicochemical properties of biological soil crusts (BSCs)

	SOC	Total N	Total P	Total K	Available N	Available P	Available K	pH	EC	Total salts	Soil moisture	Soil temperature	Irradiance
SOC	1.000												
Total N	0.817**	1.000											
Total P	0.357*	0.256	1.000										
Total K	-0.067	0.028	0.013	1.000									
Available N	0.179	0.052	0.283	-0.088	1.000								
Available P	0.490**	0.364*	0.251	-0.062	0.175	1.000							
Available K	0.661**	0.477*	0.036*	-0.264	0.127	0.370*	1.000						
pH	-0.733**	-0.525**	-0.255	0.173	-0.293	-0.297*	-0.630**	1.000					
EC	0.092	0.114	0.090	-0.125	0.575**	0.087	0.075	-0.339*	1.000				
Total salts	0.188	0.196	0.001	0.067	0.163	0.109	0.295*	-0.376*	0.024	1.000			
Soil moisture	0.199	0.270	0.254	0.258	-0.021	0.438**	0.004	0.232	-0.335*	-0.002	1.000		
Soil temperature	0.430**	0.387**	-0.087	-0.264	-0.023	0.151	0.521**	-0.624**	0.044	0.353*	-0.181	1.000	
Irradiance	0.267	0.092	-0.067	-0.301*	0.010	-0.040	0.433**	-0.636**	0.143	0.235	-0.647**	0.729**	1.000

Note: SOC, soil organic carbon; EC, electrical conductivity. ** and * indicate significant correlations at the 0.01 and 0.05 levels, respectively.

Table S2 Affiliations of cyanobacterial operational taxonomic units (OTUs) and their relative abundances in BSCs in different snow treatments and stages

Affiliations of cyanobacterial OTUs	Accession number	Identity to CR (%)	Relative abundance in stage 1 (%)			Relative abundance in stage 2 (%)			Relative abundance in stage 3 (%)		
			DS	AS	RS	DS	AS	RS	DS	AS	RS
Microcoleaceae											
<i>Microcoleus vaginatus</i>	EF654079.1	97.0–99.3	3.30±1.42 ^{Bb}	5.54±2.90 ^{Bb}	14.42±1.20 ^{AaBa}	10.57±5.68 ^{Ab}	12.32±2.80 ^{Aab}	18.93±6.02 ^{Aa}	13.68±4.05 ^{Aa}	11.23±1.88 ^{Aa}	10.40±3.41 ^{Ba}
<i>M. steenstrupii</i>	KC999639.1	93.1–99.0	0.77±0.64 ^{Ab}	1.22±0.89 ^{Aab}	2.08±1.07 ^{Aa}	1.66±1.61 ^{Ab}	2.20±1.05 ^{Aab}	3.29±1.63 ^{Aa}	1.72±0.44 ^{Aa}	2.00±0.43 ^{Aa}	2.45±0.27 ^{Aa}
<i>Trichodesmium contortum</i>	AF013028	88.9–89.8	0.39±0.29 ^{Ab}	0.77±0.55 ^{Bab}	1.62±0.86 ^{Aa}	1.14±1.28 ^{Ab}	1.42±0.72 ^{Ab}	2.60±1.49 ^{Aa}	1.20±0.33 ^{Aa}	1.60±0.15 ^{ABa}	1.47±0.33 ^{Aa}
<i>Symplacnistrum fletcherii</i>	KF312349.1	98.6	0.03±0.02 ^{Ab}	0.04±0.03 ^{Ab}	0.09±0.01 ^{Aa}	0.05±0.04 ^{Aa}	0.08±0.04 ^{Aa}	0.09±0.02 ^{Aa}	0.06±0.02 ^{Aa}	0.07±0.02 ^{Aa}	0.09±0.01 ^{Aa}
<i>S. torvium</i>	EF654065.1	96.9–98.6	0.22±0.20 ^{Ab}	0.33±0.25 ^{Aab}	0.54±0.29 ^{Aa}	0.36±0.29 ^{Ab}	0.57±0.29 ^{Aa}	0.67±0.20 ^{Aa}	0.45±0.12 ^{Aa}	0.49±0.10 ^{Aa}	0.66±0.05 ^{Aa}
<i>S. californicum</i>	KF312350.1	93.2–96.8	0.46±0.40 ^{Ab}	0.67±0.47 ^{Aab}	1.25±0.69 ^{Aa}	0.96±0.92 ^{Ab}	1.32±0.60 ^{Aab}	1.88±0.81 ^{Aa}	0.96±0.24 ^{Aa}	1.06±0.22 ^{Aa}	1.39±0.07 ^{Aa}
Phormidaceae											
<i>Phormidium murrayi</i>	DQ493872.1	97.4–97.6	0.04±0.03 ^{Ab}	0.06±0.04 ^{Ab}	0.11±0.06 ^{Aa}	0.07±0.05 ^{Aa}	0.10±0.05 ^{Aa}	0.11±0.02 ^{Aa}	0.08±0.02 ^{Aa}	0.09±0.02 ^{Aa}	0.12±0.01 ^{Aa}
Phormidaceae cyanobacterium	EF654088.1	91.8	0.01±0.01 ^{Ab}	0.01±0.00 ^{Bb}	0.02±0.01 ^{Aa}	0.01±0.01 ^{Ab}	0.02±0.01 ^{Aa}	0.02±0.00 ^{Aa}	0.02±0.00 ^{Aa}	0.10±0.00 ^{ABa}	0.02±0.00 ^{Aa}
Coleofasciculaceae											
<i>Coleofasciculus chthonoplastes</i>	EF654032.1	93.4–94.2	0.39±0.33 ^{Aa}	0.67±0.51 ^{Aa}	1.07±0.58 ^{Ba}	1.00±1.09 ^{Ab}	1.23±0.61 ^{Aab}	2.18±1.58 ^{Aa}	0.97±0.27 ^{Aa}	1.15±0.26 ^{Aa}	1.30±0.20 ^{ABa}
<i>Geitlerinema pseudocutissimum</i>	KT315938.1	90.9–94.2	0.07±0.05 ^{Ab}	0.09±0.07 ^{Ab}	0.19±0.10 ^{Aa}	0.11±0.09 ^{Aa}	0.17±0.09 ^{Aa}	0.18±0.04 ^{Aa}	0.12±0.02 ^{Aa}	0.14±0.03 ^{Aa}	0.17±0.01 ^{Aa}
Chroococcidiopsis sp. CC1											
<i>Chroococcidiopsis sp. CC1</i>	DQ914863.2	88.5–97.8	2.14±1.27 ^{Bb}	3.18±1.41 ^{Bb}	5.37±2.35 ^{Ba}	4.30±2.41 ^{Ab}	5.62±1.95 ^{Ab}	7.98±2.10 ^{Aa}	4.45±1.20 ^{ABa}	4.94±0.92 ^{Aa}	6.54±0.40 ^{Aa}
<i>Chroococcidiopsis thermalis</i>	IX494785.1	93.2–99.0	0.69±0.60 ^{Ab}	1.08±0.80 ^{Aab}	1.81±0.97 ^{Aa}	1.42±1.32 ^{Ab}	1.91±0.87 ^{Aab}	2.79±1.36 ^{Aa}	1.49±0.49 ^{Ab}	1.66±0.35 ^{Aa}	2.15±0.19 ^{Aa}
<i>Chroococcidiopsis sp. CC3</i>	DQ914865.2	93.4–93.9	0.22±0.19 ^{Ab}	0.33±0.24 ^{Aab}	0.57±0.29 ^{Aa}	0.37±0.29 ^{Ab}	0.58±0.29 ^{Aab}	0.67±0.19 ^{Aa}	0.45±0.13 ^{Aa}	0.50±0.11 ^{Aa}	0.68±0.06 ^{Aa}
Chroococcaceae											
<i>Gloeocapsa quaternata</i>	KM982577.1	87.0	0.07±0.06 ^{Ab}	0.10±0.07 ^{Aab}	0.17±0.09 ^{Aa}	0.12±0.10 ^{Aa}	0.16±0.09 ^{Aa}	0.19±0.05 ^{Aa}	0.14±0.04 ^{Aa}	0.15±0.04 ^{Aa}	0.21±0.02 ^{Aa}
Noctocaceae											
<i>Nostoc commune</i>	AB933330.2	97.3–98.8	0.10±0.09 ^{Ab}	0.14±0.09 ^{Aab}	0.26±0.13 ^{Aa}	0.17±0.14 ^{Aa}	0.24±0.13 ^{Aa}	0.27±0.06 ^{Aa}	0.19±0.05 ^{Aa}	0.22±0.05 ^{Aa}	0.19±0.02 ^{Aa}
<i>Nostoc flagelliforme</i>	EU178143.1	99.5	0.10±0.08 ^{Ab}	0.14±0.09 ^{Aab}	0.23±0.11 ^{Aa}	0.17±0.15 ^{Aa}	0.23±0.12 ^{Aa}	0.28±0.08 ^{Aa}	0.20±0.07 ^{Aa}	0.21±0.05 ^{Aa}	0.28±0.03 ^{Aa}
Seytonemataceae											
<i>Seytonema sp.</i>	HO847553	88.3–99.3	0.38±0.32 ^{Ab}	0.57±0.42 ^{Aab}	0.10±0.52 ^{Aa}	0.66±0.52 ^{Aa}	0.95±0.46 ^{Aa}	1.12±0.30 ^{Aa}	0.76±0.20 ^{Aa}	0.86±0.19 ^{Aa}	0.11±0.12 ^{Aa}
<i>Seytonema hyalinum</i>	AF334700	94.7–96.2	0.37±0.32 ^{Ab}	0.52±0.34 ^{Aab}	0.87±0.43 ^{Aa}	0.67±0.56 ^{Aa}	0.90±0.45 ^{Aa}	1.11±0.31 ^{Aa}	0.73±0.24 ^{Aa}	0.82±0.20 ^{Aa}	1.10±0.11 ^{Aa}
Tolypothrixaceae											
<i>Tolypothrix distorta</i>	AF334694	98–98.5	0.08±0.06 ^{Ab}	0.12±0.08 ^{Ab}	0.22±0.11 ^{Aa}	0.14±0.11 ^{Aa}	0.20±0.10 ^{Aa}	0.23±0.05 ^{Aa}	0.16±0.05 ^{Aa}	0.18±0.04 ^{Aa}	0.24±0.02 ^{Aa}
<i>Spirirestis rafaensis</i>	AF334692	99.0	0.06±0.04 ^{Ab}	0.08±0.06 ^{Aab}	0.15±0.08 ^{Aa}	0.09±0.07 ^{Aa}	0.13±0.06 ^{Aa}	0.16±0.03 ^{Aa}	0.11±0.03 ^{Aa}	0.12±0.03 ^{Aa}	0.17±0.02 ^{Aa}
Leptolyngaceae											
<i>Trichocoleus sociatus</i>	EF654080.1	98.0	0.14±0.13 ^{Ab}	0.20±0.14 ^{Ab}	0.39±0.20 ^{Aa}	0.24±0.19 ^{Aa}	0.36±0.19 ^{Aa}	0.39±0.19 ^{Aa}	0.28±0.07 ^{Aa}	0.31±0.07 ^{Aa}	0.43±0.01 ^{Aa}
Unknown cyanobacteria											
			0.67±0.51 ^{Bb}	1.21±0.78 ^{Ab}	3.25±0.47 ^{Aa}	2.50±1.02 ^{Aa}	2.54±1.02 ^{Aa}	3.68±1.65 ^{Aa}	3.18±1.43 ^{Aa}	2.27±0.28 ^{Aa}	2.16±0.65 ^{Aa}

Note: CR, closest relative; stage 1, snowpack; stage 2, melting snow; stage 3, melted snow; DS, double snow; AS, ambient snow; RS, removed snow. The values are means±standard deviations. The values labeled with different lowercase letters in the same row indicate significant differences at the $P<0.05$ level among different snow treatments in same stage (LSD), and those labeled with different uppercase letters in the same row indicate significant difference at the $P<0.05$ level (LSD) in different stages of same snow treatment.